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Safety and efficacy of Feedlyve AXC (endo-1,4-β-xylanase) as a feed additive for chickens for fattening

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Abstract

Feedlyve AXC is a feed additive that is available in liquid and solid formulations and contains endo-1,4-β-xylanase which is produced by a strain of *Trichoderma longibrachiatum*. The tolerance trial submitted did not comply with the requirements of tolerance trials, and therefore, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of the additive for the target species. The enzyme concentrate gave negative results in a bacterial reverse mutation assay and in an *in vitro* chromosome aberration assay. Moreover, the substance was negative in an *in vivo* micronucleus test in the rat bone marrow and the results of the subchronic oral toxicity study showed no adverse effects. However, the correspondence of the test item used and the fermentation product that is currently used to prepare the additive was not established. Therefore, the FEEDAP Panel was not in a position to conclude on the safety for the consumer of the additive. The tests conducted in order to address the safety for the user indicated that the test items were not toxic by inhalation, not irritant to the eyes or skin but showed a dermal sensitisation potential. However, the relationship between the test items and the additive for which re-evaluation is sought were not fully established. The additive is considered as a potential respiratory sensitiser. No risks to the environment are expected. The results of five trials showed that 100 AXC U/kg feed increased the content of apparent metabolisable energy of the diets in one trial, and 80 AXC U/kg feed improved the feed to gain ratio in two other trials. The trials providing evidence of efficacy at 80 AXC U/kg feed showed some limitations, and therefore, the Panel considered that there is not sufficient data to conclude on the efficacy of the additive.

Keywords: zootechnical additives, digestibility enhancers, safety, efficacy, xylanase, chickens

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Feedlyve AXC as a feed additive for chickens for fattening. The applicant requested for the re-evaluation of the use of this additive in chickens for fattening. Feedlyve AXC is a preparation of endo-1,4-β-xylanase (xylanase) that is available in liquid and solid formulations. The enzyme that is contained in the additive is produced by a strain of *Trichoderma longibrachiatum*. However, the identity of the strain was not confirmed by molecular techniques.

One tolerance trial in chickens for fattening was evaluated. The animals were under study from day 7 to day 21 of life. This duration was not in accordance with the requirements for tolerance trials in terms of age at start and duration of the treatment (start on day 1 of life and study for 35 days). Moreover, the study lacked replicates, and therefore, the statistical evaluation of the data would not be possible. The Panel considered this study as not valid and could not conclude on the safety of the additive for the target species.

The enzyme concentrate gave negative results in a bacterial reverse mutation assay and in an *in vitro* chromosome aberration assay. Moreover, the substance was negative in an *in vivo* micronucleus test in the rat bone marrow, although no evidence of target cell exposure was provided. The results of a subchronic oral toxicity study showed no adverse effects. These tests were conducted in the 1990s, with the exception of the *in vivo* micronucleus test, which was performed in 2010. The correspondence of the test item used and the fermentation product that is currently used to prepare the additive was not established. Therefore, the FEEDAP Panel was not in a position to conclude on the safety for the consumer of the additive.

An acute inhalation study, the skin and eye irritation tests and a skin sensitisation study were performed and indicated that the test items were not toxic by inhalation and not irritant to the eyes or skin but had a dermal sensitisation potential. However, the relationship between the test items and the additive for which re-evaluation is sought were not fully established. Owing to the proteinaceous nature of the active substance, the additive is considered as a potential respiratory sensitiser.

The active substance of the additive is a protein and as such will be degraded/inactivated during the passage through the digestive tract of animals. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

Six efficacy trials, two short-term and four long-term, were submitted. One of the short-term trials was not considered further due to the nature of the parameters measured. The results of the other five trials showed that the addition of 100 AXC U/kg feed increased the content of apparent metabolisable energy of the diets in one trial, and the addition of 80 AXC U/kg feed improved the feed to gain ratio in two other trials. The trials providing evidence of efficacy at 80 AXC U/kg feed showed some limitations; in one, the analytical confirmation of the intended dosage was not provided, and in the other one, it was not confirmed that the statistical analysis was done on pen basis. Therefore, the Panel considered that there is not sufficient data to conclude on the efficacy of the additive.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest 1 year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from LYVEN[2] for re-evaluation of the authorisation of the product Feedlyve AXC (endo-1,4-β-xylanase), when used as a feed additive for chickens for fattening (category: zootechnical additive; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 16 April 2015.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment, and on the efficacy of the product Feedlyve AXC (endo-1,4-β-xylanase), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The Scientific Committee on Animal Nutrition issued an opinion on the safety of use of this additive for animals, consumers and users (EC, 1996, updated in 2002). The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and the efficacy of the product when used as a feed additive for turkeys for fattening or reared for breeding (EFSA FEEDAP Panel, 2012a).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of Feedlyve AXC as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[4] and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Feedlyve AXC is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012b), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] LYVEN, ZAC de Normandial, 11 Avenue de Pays de Caen, 14460 Colombelles, France.
[3] FEED dossier reference: FAD-2010-0213.
[4] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0213-feedlyve_axc.pdf

assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

This opinion deals with the re-evaluation of the safety and efficacy of Feedlyve AXC (endo-1,4-β-xylanase (EC 3.2.1.4; xylanase)) as a feed additive for chickens for fattening.

3.1. Characterisation

3.1.1. Characterisation of the active substance

The main activity of the additive is xylanase but other side activities are present (non-starch polysaccharidases). The xylanase is obtained by fermentation with a strain of *Trichoderma longibrachiatum* which was deposited at the Mycothèque de l'Université Catholique de Louvain with the accession number MUCL 39203. No evidence was provided that the strain is still deposited in the culture collection.[6] The identification of the strain was carried out by morphological examination,[7] but no confirmation of the identity was provided using molecular techniques. Characteristics of the growth of the strain and ability to produce xylanase were provided along with the genomic fingerprinting of the strain.[8] This strain is a mutant, as stated in the certificate of deposition, but no details on the methodologies and steps followed to select the strain were provided in order to support the statement. The genetic stability of the strain was not studied and no data on the potential of the production strain to produce potentially toxic secondary metabolites was provided.

3.1.2. Manufacturing process[9]

The enzyme is obtained by a multi-step process consisting of fermentation, concentration and purification steps. The product obtained is stabilised. The liquid formulations are obtained from this product by adding carriers and stabilisers. For the preparation of the solid formulations, the stabilised liquid is spray-dried. The spray-dried product (referred to as Feedlyve AXC BRUTE (CNS)) is used to prepare the solid formulations. Indications on the enzyme activity at the different points of the manufacturing were provided, expressed per initial dry matter in the fermenter.

The dossier contains information on the spray-dried product used to prepare the solid formulations, Feedlyve AXC BRUTE (CNS). The batch-to-batch variation in the enzyme activity in three batches from 1993 to 1995 showed mean values of 17,456 AXC U/g (range: 12,500–21,269).[10,11]

3.1.3. Characterisation of the additive

The additive is available in solid (Feedlyve AXC 1500 P and 6000 P) and liquid forms (Feedlyve AXC 200 L and 1500 L). Two other solid formulations, Feedlyve AXC 400 P and 12000 P, have been mentioned in the dossier but the information does not allow a full characterisation of these formulations.

Feedlyve AXC 1500 P and 6000 P are the solid formulations standardised to ensure 1,500 or 6,000 AXC U/g, respectively. The study of the batch-to-batch variation in five batches showed a mean value of 1,869 AXC U/g (range: 1,659–2,300 with a coefficient of variation (CV) of 14%) for Feedlyve AXC 1500 P and a mean value of 7,162 AXC U/g (6,208–8,304; CV of 11%) for Feedlyve AXC 6000 P.[12] These formulations contain Feedlyve AXC BRUTE (CNS), 0.5% tricalcium phosphate and wheat flour. Data on the physicochemical properties of the two solid formulations were submitted, including pH, density, particle size distribution and dusting potential.[11] The study of the particle size (by sieving) in three batches of each formulation was provided and the particles < 40 μm ranged from 53% to 56% for Feedlyve AXC 1500 P and from 47% to 61% for Feedlyve AXC 6000 P, but the data did not include

[6] Technical dossier/Section II/Annex II.2.1.B.
[7] Technical dossier/Section II/Annex II.2.1.C and II.2.1.D.
[8] Technical dossier/Section II/Annex II.2.1.E–2.1.G and Annex II.2.1.Fbis.
[9] This section has been amended following the confidentiality claims made by the applicant.
[10] AXC U is the amount of enzyme which liberates 17.2 μmol of reducing sugars (xylose equivalents) from oat xylan per minute at pH 4.7 and 30°C.
[11] Technical dossier/Section II/Annex II.1.5.B.
[12] Technical dossier/Section II/Annex II.1.3.A.

information on particle size distribution below this value. The dusting potential was measured in one batch for each formulation and was 0.6 and 55 mg/50 g additive, respectively.[13]

Feedlyve AXC 200 L and 1500 L are the liquid formulations standardised to ensure 200 or 1,500 AXC U/g, respectively. The study of the batch-to-batch variation in five batches showed a mean value of 249 AXC U/g (range 244–255; CV of 2%) for Feedlyve AXC 200 L and a mean value of 1,570 AXC U/g (1,350–1,700; CV of 9%) for Feedlyve AXC 1500 L.[12] These formulations contain the liquid product obtained after fermentation, 30% sorbitol, 5% monopotassium phosphate, 0.02% potassium sorbate and water.[14] The pH of these formulations is 4 and the density is 1.18–1.20 kg/m^3.[11]

The purity of the final formulations was not provided; however, data on one batch of the fermentation product (liquid) was made available for chemical and microbial purity.[15] Chemical analysis included lead (0.10 mg/kg), arsenic (< 0.10 mg/kg), cadmium (< 0.05 mg/kg) and mercury (< 0.03 mg/kg). Mycotoxins including aflatoxins (< 0.1 μg/kg), zearalenone (6.0 μg/kg) and ochratoxin (0.5 μg/kg) were also analysed in these batches. Microbial analysis was done for total viable counts (< 10,000 colony-forming units (CFU)/g), *Salmonella* spp. (absent in 25 g), coliforms, *Escherichia coli* (< 10 CFU/g), anaerobic sulfate reducers (< 10 CFU/g) and *Staphylococcus aureus* (absent in 1 g). The test on the presence of antibiotic activity revealed no evidence of such activity. The same information (including also the absence of the production strain) was provided for three batches of Feedlyve AXC BRUTE (CNS),[16] batches from 1993 to 1995, but the correspondence of the batches analysed with the product that is currently used to manufacture the additive has not been established.

3.1.4. Stability and homogeneity

3.1.4.1. Shelf life

Three batches of the solid (1500 P, 6000 P and BRUTE) and liquid formulations (200 L and 1500 L) were kept at three different temperatures (4, 20 and 30°C) in closed packages for 3 months (liquid) or for 12 months (solid).[17] The enzyme recoveries were expressed as the % of activity present in the sample compared to the activity present in a frozen sample. After 3 months, the recoveries in the liquid formulations stored at 4, 20 or 30°C were 98%, 87% and 70% for Feedlyve AXC 200 L and 100%, 90% and 71% for Feedlyve AXC 1500 L. After 12 months, the recoveries in the solid formulations were > 95% in all cases. The test also included the study of the effect of light, by comparing samples in darkness and the effect of air and relative humidity (for the solid formulations).[18] These factors had no impact on the shelf life of the additive.

The data provided showed the following limitations: the claimed shelf life (2 years) was not studied in any of the tests, the initial enzyme activity of the batches was not provided and the recoveries were calculated comparing the enzyme activity to a frozen sample, which could have also lost activity.

3.1.4.2. Stability and homogeneity

The stability of three batches of each solid formulation (1500 P and 6000 P), when added to a complete vitamin–mineral premixture (without choline chloride) at an enzyme activity of 25 U/g, was studied in samples kept at 4, 20 and 30°C for up to 4 months.[19] After 4 months, mean enzyme activity was > 93% in all cases (expressed as compared to a frozen sample). No data for 6 months storage was available.

The stability of the additive (unknown formulation) to processing was studied when added to a compound feed and then pelleted at 75, 78 or 80°C.[20] Recoveries were < 33% showing a low stability to pelleting. The stability of three batches of the liquid (200 L and 1500 L) and solid formulations (1500 P and 6000 P), when added to a complete compound feed (mash form) at an enzyme activity of 100 AXC U/kg feed, was studied in samples kept at 4, 20 and 30°C for up to 3 months.[21] Mean recovery values were > 93% (expressed as compared to a frozen sample). Premixture and mash feed used for the stability studies were sampled five times (less than the required 10 subsamples) in order to study the

[13] Technical dossier/Section II/Annex II.1.5.C.
[14] Technical dossier/Section II/Annex II.1.3.
[15] Technical dossier/Section II/Annex II.2.1.J.
[16] Technical dossier/Section II/Annex II.1.4.A–1.4.E.
[17] Technical dossier/Section II/Annex II.4.1.A.
[18] Technical dossier/Section II/Annex II.4.1.B, II.4.1.C and II.4.1.D.
[19] Technical dossier/Section II/Annex II.4.1.E and Annex II.4.1.J.
[20] Technical dossier/Section II/Annex II.4.1.H and Annex II.4.1.F.
[21] Technical dossier/Section II/Annex II.4.1.K and Annex II.4.1.F.

capacity of the additive to distribute homogeneously.[22] Samples in premixtures showed a CV < 6% and samples in feed showed CV of 6% for the solid formulations and < 12% for the liquid formulations.

3.1.5. Conditions of use

The conditions of use of the additive were defined as to be used in chickens for fattening up to 35 days of life at a minimum dose of 55 AXC U/kg feed and a maximum dose of 100 AXC U/kg feed.

3.2. Safety

3.2.1. Safety for chickens for fattening

The applicant submitted one trial in chickens for fattening to support the safety for the target sepcies.[23] However, this study is considered not acceptable since the animals were under study from day 7 to day 21 of life, and there were no replicates. Therefore, the Panel cannot conclude on the safety of the additive for the target species.

3.2.2. Safety for the consumer

The applicant submitted a bacterial reverse mutation assay (OECD TG 471),[24] an *in vitro* chromosome aberration assay (OECD TG 473),[25] an *in vivo* micronucleus test (OECD TG 474)[26] and a subchronic oral toxicity study (OECD TG 408).[27]

The test item used for these studies was Feedlyve AXC BRUTE (CNS) which was claimed to be the non-standardised spray-dried fermentation product with dextrin and monopotassium phosphate used in the formulation of the solid formulations. The tests items used in these tests were manufactured in the 1990s with the exception of the one used in the *in vivo* micronucleus test, that was from 2010. The correspondence of those test items and the fermentation product that is currently used to formulate the additive was not established.

The results obtained in the studies submitted do not indicate any reason for concern for consumer safety arising from the use of the additive as a feed additive. However, since the correspondence of the test item and the fermentation product that is currently used has not been established, the FEEDAP Panel is not in a position to conclude on the safety for the consumer of the additive.

3.2.3. Safety for the user

An acute inhalation study (OECD TG 403),[28] the skin (OECD TG 404) and eye (OECD TG 405) irritation studies[29] and a skin sensitisation study (OECD TG 406).[30] were submitted. Feedlyve AXC BRUTE (CNS) was the test item in the acute inhalation study and in the skin sensitisation study, and a test item identified as 'Xylanase 6400' was used in the skin and eye irritation studies.

The test materials were not toxic by inhalation and not irritant to the skin and eyes, but showed a moderate dermal sensitising potential. However, their correspondence with the product for which re-evaluation is sought was not established; therefore, the FEEDAP Panel is not in the position to conclude on these properties. Owing to the proteinaceous nature of the active substance, the additive is considered as a potential respiratory sensitiser.

3.2.4. Safety for the environment

The active substance of the additive is a protein and as such will be degraded/inactivated during the passage through the digestive tract of animals. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

[22] Technical dossier/Section II/Annexes II.4.2.A–4.2.C.
[23] Technical dossier/Section III/Annex III.1.A.
[24] Technical dossier/Section III/Annex III.2.2.B.
[25] Technical dossier/Section III/Annex III.2.2.C.
[26] Technial dossier/Section III/Annex III.2.2.H.
[27] Technical dossier/Section III/Annexes III.2.2.D–2.2.G.
[28] Technical dossier/Section III/Annex III.2.2.A.
[29] Technical dossier/Section III/Annex III.3.1.B and III.3.1.A.
[30] Technical dossier/Section III/Annex III.3.1.C.

3.3. Efficacy

Six trials, two short-term and four long-term, were submitted. One of the short-term trials was not considered further due to the nature of the parameters measured.[31]

In the short-term trial considered,[32] a total of thirty-six 14-days-old male chickens for fattening were distributed in groups of three birds to two dietary treatments (representing six replicates per treatment). A basal diet based on wheat and soya bean meal was supplemented or not with Feedlyve AXC 1500 P to provide 0 or 100 AXC U/kg feed (confirmed by analysis). The diets were offered to the birds for 7 days in mash form and contained titanium dioxide as an external marker. On day 21, samples of the excreta were collected and analysed for gross energy, nitrogen and the marker. The apparent metabolisable energy (corrected for nitrogen) of the diets was calculated. Comparison of the results was done with a t-test. Results showed that the apparent metabolisable energy contained in the diets supplemented with the enzyme was higher than the non-supplemented (from 10.89 to 11.33 MJ/kg feed).

The four long-term trials shared a very similar study design. Details on the number of animals and treatments considered are presented in Table 1. One-day-old chickens were used in the four studies; males in trials 1, 2 and 4, and males and females (50:50) in trial 3. The birds received the experimental diets for more than 37 days. In these studies, the basal diets were based on wheat and soya (composition not given for trial 3) and were either not supplemented, control diet, or supplemented with Feedlyve 1500 P to provide dosages from 80 to 200 AXC U/kg feed (see Table 1). The dosages were confirmed with the exception of trial four, for which the analytical results were not given. Diets in trial 3 contained antibiotics as growth factors. In all the studies, health status and mortality were checked, body weight and feed intake were measured during the study and feed to gain ratio was calculated. Other parameters measured included water consumption, incidence of vent pasting, digesta viscosity, counts of viable microorganisms, coliforms and lactobacilli in the excreta and carcass characteristics (these data were considered as not supportive of the efficacy and therefore are not shown). The data of the individual studies were statistically analysed with an analysis of variance. In principle, the analysis was done considering the values on pen basis but this was not confirmed for trials 2 and 3. The results are shown in Table 2; data showed that the additive at the intended dose of 80 AXC U/kg feed improved the feed to gain ratio in trials 3 and 4.

Table 1: Trial design and dosages of the efficacy trials performed in chickens for fattening

Trial	Total N Animals (animals/replicate) Replicates/treatment	Breed Duration Sex	Diet composition	Intended enzyme activity (AXC U/kg feed)
1[(a)]	210 (14) 5	Cobb 42 days ♂	Wheat, soya bean meal	0 100 200
2[(b)]	360 (30) 6	Cobb 42 days ♂	Wheat, soya bean meal	0 100
3[(c)]	1,239 (~ 100) 6	Ross 37 days ♀/♂	Wheat	0 80
4[(d)]	630 (45) 7	Ross 308 39 days ♂	Wheat, soya bean meal	0 80

(a): Technical dossier/Section IV/Annex IV.3.A.
(b): Technical dossier/Section IV/Annex IV.3.B.
(c): Technical dossier/Section IV/Annex IV.3.C.
(d): Technical dossier/Section IV/Annex IV.3.D.

[31] Technical dossier/Section IV/Annex IV.2.B.
[32] Technical dossier/Section IV/Annex IV.2.A.

Table 2: Effects of Feedlyve AXC on the performance of chickens for fattening

Trial	Treatments	Feed intake (g)(1)	Weight gain (g)(2)	Feed to gain ratio	Mortality and culling (%)
1	0	92.5	47.5	1.99	4.2
	100	92.1	48.3	1.91	2.8
	200	90.7	49.4	1.88	5.7
2	0	4,380	1,958	2.23	3.9
	100	4,478	2,035	2.20	2.8
3	0	3,110	46.0	1.81[a]	2.8
	80	3,062	46.2	1.76[b]	4.0
4	0	98.4[a]	2,121	1.81[a]	2.0
	80	92.4[b]	2,144	1.68[b]	0.7

(1): In trial 1 and 4, the data show daily feed intake, and in trial 2 and 3, overall feed intake.
(2): In trial 1 and 3, the data show daily weight gain, and in trial 2 and 4, overall weight gain.
a,b: Within a column and within a trial, values with a different superscript are significantly different ($p < 0.05$).

The dose of 55 AXC/kg feed was not tested in any of the trials. The addition of 100 AXC U/kg feed increased the content of apparent metabolisable energy of the diets in one trial, and the addition of 80 AXC U/kg feed improved the feed to gain ratio in two other trials. However, considering the limitations of the studies providing evidence of efficacy at 80 AXC U/kg feed (lack of analytical confirmation, basis for the statistical analysis not given), the FEEDAP Panel considers that there is insufficient evidence to conclude on the efficacy of the additive.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[33] and Good Manufacturing Practice.

4. Conclusions

Owing to the lack of data/information, the FEEDAP Panel is not in the position to conclude on the identity of the production strain, the safety of the additive for the target species, consumer or on the dermal/eye irritancy potential and the dermal sensitisation properties of the additive. The additive is to be considered a potential respiratory sensitiser. The use of Feedlyve AXC as a feed additive poses no risks to the environment.

The Panel cannot conclude on the efficacy of Feedlyve AXC as a feed additive for chickens for fattening.

Documentation provided to EFSA

1) Feedlyve AXC for chickens for fattening. November 2010. Submitted by LYVEN.
2) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Feedlyve AXC.
3) Comments from Member States.

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[33] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. doi:10.2903/j.efsa.2011.2175

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Abbreviations

CFU	colony-forming unit
CV	coefficient of variation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
MUCL	Mycothèque de l'Université Catholique de Louvain
OECD	Organisation for Economic Co-operation and Development
TG	Technical Guideline

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Feedlyve AXC

Feedlyve® AXC is currently authorised as feed additive for chickens for fattening by Commission Regulation (EC) No 828/2007 and for turkeys by Commission Implementing Regulation (EC) No 1195/2012. In the current application an authorisation is sought under article 10 (2) of the Regulation (EC) No 1831/2003 under the category/functional group "zootechnical additives"/ "digestibility enhancers" for chickens for fattening.

According to the Applicant, Feedlyve® AXC contains endo-1,4-β-xylanase as active agent. The product is intended to be marketed as solid and liquid formulations having a guaranteed minimum xylanase activity ranging from 200 to 20000 AXC/g of product. The feed additive formulations are intended to be included through premixtures or directly in feedingstuffs to obtain a minimum activity of 55 AXC/kg feedingstuffs. However the Applicant proposed a recommended dose ranging from 55 to 100 AXC/kg feedingstuffs. The Applicant expresses the xylanase enzymatic activity in xylanase units (AXC), defined as "1 AXC, the amount of enzyme which liberates 17.2 micromoles of reducing sugars (xylose equivalents) from oat xylan per minute at pH 4.7 and 30 °C".

For the quantification of the xylanase activity in the feed additive and feedingstuffs the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the enzymatic hydrolysis of oat spelt xylan at pH 4.7 and 30 °C and the quantification of dyed oligomers released from the substrate Remazol-Brilliant-Blue-R xylan. Furthermore, the Applicant applied this method successfully for the quantification of xylanase in premixtures, in the frame of the stability studies. Based on the satisfactory performance characteristics available, the EURL recommends for official control the colorimetric method for the quantification of the xylanase activity in the feed additive, premixtures and feedingstuffs.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.